Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached is captioned "**VERSION WITH MARKINGS TO SHOW CHANGES MADE.**"

Applicants respectfully request reconsideration of the application in view of the remarks made herein.

Rejection under 35 U.S.C. § 102(b)

Claims 1, 15 and 16 have been rejected under 35 U.S.C. §102(b) as being anticipated by Grumetto.

Claims 1, 15 and 16 as amended, are directed towards a method of activating an oocyte *in vitro*, wherein the oocyte is exposed to a modulator of NO levels and the oocyte undergoes at least one cell division. A limitation of each of these claims is that the method must teach an oocyte that undergoes at least one cell division.

In making the above rejection, the Office asserts that Grumetto discloses methods for modulating activation of oocytes comprising the step of contacting *in vitro* cultured oocytes from ascidia with modulators of nitric oxide levels such as NO doners and/or inhibitors of NO synthase in the absence of sperm. However, Grumetto does not recite a method wherein the oocyte undergoes at least one cell division.

Since the subject claims are directed to a method that teaches oocytes that undergo at least one cell division, the subject claims are not anticipated by the Grumetto reference.

Since Claim 16 is now cancelled, the rejection of Claim 16 is now moot

Applicants submit that the rejection of claims 1, 15 and 16 under 35 U.S.C. 102(b) has been adequately addressed in view of the remarks set forth above. The Examiner is thus respectfully requested to withdraw the rejection.

Claims 1, 3, 5, 15 and 16 have been rejected under 35 U.S.C. §102(b) as being anticipated by Jawerbaum.

Claims 1, 3, 15 and 16, as amended, are directed towards a method of activating an oocyte *in vitro*, wherein the oocyte is exposed to a modulator of NO levels and the oocyte

undergoes at least one cell division. A limitation of each of these claims is that the method must teach an oocyte that undergoes at least one cell division.

In making the above rejection, the Office asserts that Jawerbaum discloses methods for modulating activation of oocytes comprising the step of contacting *in vitro* cultured oocytes from rats with modulators of nitric oxide levels such as NO donors and/or inhibitors of NO synthase in the absence of sperm. However, Jawerbaum does not recite a method wherein the oocyte undergoes at least one cell division.

Therefore, Jawerbaum does not disclose a method that teaches oocytes that undergo at least one cell division. Since the subject claims are directed to a method that teaches oocytes that undergo at least one cell division, the subject claims are not anticipated by the Jawerbaum reference.

Since Claim 16 is now cancelled, the rejection of Claim 16 is now moot.

Applicants submit that the rejection of claims 1, 3, 15 and 16 under 35 U.S.C. 102(b) has been adequately addressed in view of the remarks set forth above. The Examiner is thus respectfully requested to withdraw the rejection.

Claim 5 has been rejected under 35 U.S.C. §102(b) as being anticipated by Jawerbaum.

Claims 5, as amended, is directed towards a method of inhibiting the activation of an oocyte during fertilization *in vitro*, wherein the oocyte is exposed to the inhibitor prior to, during, or after the oocyte is contacted with sperm.

In making the above rejection, the Office asserts that Jawerbaum discloses methods for inhibiting activation of rat oocytes containing the step of contacting *in vitro* cultured oocytes from rats with an inhibitor of nitric oxide synthesis. However, and as the Examiner points out, Jowerbaum teaches a method in the absence of sperm.

Therefore, Jawerbaum does not disclose a method of inhibiting the activation of an oocyte during fertilization *in vitro*, wherein the oocyte is exposed to the inhibitor prior to, during, or after the oocyte is contacted with sperm. Since the subject claims are directed to a method of inhibiting the activation of an oocyte during fertilization *in vitro*, wherein the oocyte is exposed to the inhibitor prior to, during, or after the oocyte is contacted with sperm, the subject claims are not anticipated by the Jawerbaum reference.

Applicants submit that the rejection of Claim 5 under 35 U.S.C. 102(b) has been adequately addressed in view of the remarks set forth above. The Examiner is thus respectfully requested to withdraw the rejection.

Rejection under 35 U.S.C. § 103(a)

Claims 1, 3-5, 13, 15 and 16 have been rejected under 35 U.S.C. §103(a) as being obvious over Grumetto in view of Jawerbaum and US Patent 6,077,710 for the asserted reason that Grumetto and Jawerbaum recite methods for modulating activation of oocytes by contacting the oocytes with modulators of NO levels, which, when coupled with Grumetto's teaching that a Ca^{2+} increase is associated with NO levels in ascidian oocytes, and US Patent 6,077,710 which teaches that activation of mammalian oocytes can be achieved by Ca^{2+} and kinase inhibitor application, renders the claims obvious to one of skill in the art.

The M.P.E.P. teaches at §1242 that:

> To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, whether in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations.

In other words, in order for a proper *prima facie* case to be made, a reference or a combination of references must teach or suggest all of the claim limitations, there must be a motivation to combine the references, and there must be some expectation of success in combining the references.

As will be demonstrated below, the references cited in the Office Action firstly do not provide any motivation to combine and secondly cannot be combined with any expectation of success. In the argument below, a discussion of why no motivation to combine is found in the references or otherwise available to one of skill in the art, followed by a discussion of why the references cannot be combined with any expectation of success, noting that one of the references actually teaches away from the invention.

The present invention is based on the finding that NOS and NO activity are not only necessary but also sufficient for activation of an oocyte during the process of fertilization in mammals. The invention provides a straightforward and general method for preventing the activation of mammalian oocytes when necessary, or activating oocytes during the process of fertilization. The results were surprising, and have far reaching utility in human assisted reproduction, *in vitro* fertilization, contraception, nuclear transfer, whole animal cloning and animal breeding. As testament to the importance and unobviousness of the findings to the scientific community, they were published in Nature, a highly prestigious journal.

As discussed above, Grumetto discloses the addition of sodium nitroprusside, an NO donor, to the ascidian *Ciona intestinalis* oocytes. The paper further describes that the NO donor induces an inward current, a release of intracellular calcium, and show that NO production increases at fertilization. The paper also *speculates* that sperm may trigger the release of NO, and this NO may induce the inward current. The Grumetto reference fails to teach oocyte activation, and fails to teach an oocyte that undergoes at least one cell division.

Jawerbaum teaches a method of contacting an rat oocyte with NO donors and NOS inhibitors and teaches that NO can induce various prostaglandins in rat oocytes, cultured *in vitro*. Jawerbaum fails to teach inhibition of activation during fertilization, the activation of an oocyte, and fails to teach an oocyte that undergoes at least one cell division.

U.S. Patent No. 6,077,710 teaches a method of parthenocarpic activation of mammalian oocytes which recites treating the oocytes in culture with Ca2+ free cations, such as ionomycin, to the oocyte and preventing phosphorylation with serine/threonine kinase inhibitors such as DMAP. This reference, although teaching parthenocarpic oocyte activation in mammals, fails to teach contacting the oocytes with sperm, and fails to teach or suggest NO donors and inhibitors.

Applicants assert that there is no motivation to combine the references, either provided by the references themselves or available to one of skill in the art. There is no motivation in any of the references to use a modulator of NO levels in the Ca^{2+}-based method described in US Patent 6,077,710 to activate oocytes, and conversely there is no motivation in any of the references to use the methods of Grumetto or Jawerbaum to activate oocytes with NO. Furthermore, one of skill in the art would not have been motivated, until the publication of the Applicant's Nature

paper, to combine the references because the link between Ca^{2+}, NO and oocyte activation in mammals, causative or otherwise, had not been made in any organism. Furthermore a large evolutionary distance exists betwcen the ascidia (commonly known as the sea squirts) and mammals. Apart from looking very different, ascidia have very different lifestyle, physiology and reproductive strategy. Because of the obviously significant differences between ascidia and mammals, one of skill in the art would not have found a motivation to combine the references.

Applicants further assert that the references cannot be combined with any expectation of success, for several reasons. Firstly, no clear link between NO (Grumetto and Jawerbaum), Ca^{2+}, (Grumetto and US Patent 6,077,710) and oocyte activation in mammals (US Patent 6,077,710) had been made at the time at which the instant application was filed. A link would suggest that NO and Ca^{2+} are interchangeable in activating oocytes, and since no link exists, no expectation of success could have existed. As the Office Action points out, a relationship between NO and prostaglandin E synthesis in oocytes is made in Jawerbaum, however, this information does not make it obvious to one of skill in the art that NO and Ca^{2+} are interchangeable in activating oocytes. Secondly, because Ca^{2+} is a signaling molecule used only a fraction of biological signal transduction pathways, and it is not obvious which pathways are also regulated by NO, it would not have been obvious that NO could be interchanged with Ca^{2+} with any degree of expectation of success to activate mammalian oocytes. In this respect, the use of NO to activate oocytes is actually taught away by Grumetto, which states (on p721, at the bottom of first column) that "production of the inward current by SNP is not sufficient for oocyte activation", meaning that his method, which recites contacting ascidian oocytes with NO, does not activate oocytes. Thirdly, because ascidia (ie sea squirts) and mammals, although both in the phylum Chordata, are very highly diverged evolutionarily, and have very different lifestyles, biology and reproductive strategies, there would be no expectation that the effects of NO in ascidia oocytes would be reproducible in mammalian oocytes. The first indication that NO could be a universal activator of oocytes comes from the Nature paper published by the applicants, after the filing data of this application. Finally, at the time the application was filed, it was known that NO was involved in a number of processes and signal transduction pathways, but there was no indication that NO was a regulator of oocyte activation in mammals. Again, it was not until the Nature paper of the Applicants that such a relationship was taught.

In sum, the references used to reject the instant claims cannot be properly combined to teach the instant invention, because there is firstly no motivation to combine the references, and secondly there is no reasonable expectation that the combination would be successful. As such, the references, together or in combination, do not teach methods wherein an oocyte is contacted with a modulator of NO levels and then undergoes at least one cell division. As such Claims 1, 3-5, 13 and 15 are not obvious over the cited references.

Since Claim 16 is now cancelled, the rejection of Claim 16 is now moot.

Applicants submit that the rejection of claims 1, 3-5, 13, 15 and 16 under 35 U.S.C. § 103(a) has been adequately addressed in view of the remarks set forth above. The Examiner is thus respectfully requested to withdraw the rejection.

III. CONCLUSION

Applicants submit that all of the claims are in condition for allowance, which action is requested. If the Examiner finds that a telephone conference would expedite the prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

The Commissioner is hereby authorized to charge any underpayment of fees associated with this communication, including any necessary fees for extensions of time, or credit any overpayment to Deposit Account No. 50-0815, order number STAN209.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: March 26, 2002

By: James S Keddie
James S. Keddie
Registration No. 48,920

BOZICEVIC, FIELD & FRANCIS LLP
200 Middlefield Road, Suite 200
Menlo Park, CA 94025
Telephone: (650) 327-3400
Facsimile: (650) 327-3231

F:\DOCUMENT\UCAL\209\Response to final office action.doc

VERSION WITH MARKINGS TO SHOW CHANGES MADE

1. (twice amended) A method of activating an oocyte *in vitro*, the method comprising:

contacting said oocyte with nitric oxide (NO), an NO donor, nitric oxide synthase (NOS), or inducer of NOS; and,

maintaining said oocyte until the oocyte has undergone at least one cell division,

wherein said activation is performed in the absence of sperm and wherein said maintaining step indicates that the oocyte is activated ~~to reenter the mitotic cycle~~.

5. (amended) A method of inhibiting oocyte activation during fertilization *in vitro*, the method comprising:

contacting said oocyte with a nitric oxide synthase inhibitor ~~prior to or during fertilization~~; and

contacting said oocyte with sperm,

wherein said oocyte is inhibited from activation during fertilization *in vitro* ~~and reentry into the mitotic cycle~~.

15. (twice amended) ~~The method of Claim 1, further comprising the step of~~ A method of activating an oocyte *in vitro*, the method comprising:

contacting said oocyte with nitric oxide (NO), an NO donor, nitric oxide synthase (NOS), or inducer of NOS; ~~and,~~

contacting said oocyte with sperm; and ~~prior to or during said activation~~.

maintaining said oocyte until the oocyte has undergone at least one cell division,

wherein said maintaining step indicates that the oocyte is activated ~~to reenter the mitotic cycle~~.

Claim 16 is cancelled.